Franco SC, Aldred N, Sykes AV, Cruz T, Clare AS.

The effects of rearing temperature on reproductive conditioning of stalked barnacles (*Pollicipes pollicipes*).

***Aquaculture* 2015, 448, 410-417.**

THE EFFECTS OF REARING TEMPERATURE ON REPRODUCTIVE CONDITIONING OF STALKED BARNACLES (*POLLICIPES POLLICIPES*)

Sofia C. Franco[a,b,*], Nick Aldred[a], António V. Sykes[c], Teresa Cruz[b,d], Anthony S. Clare[a]

[a]School of Marine Science and Technology, Newcastle University, Newcastle upon Tyne, United Kingdom.

[b]Departamento de Biologia, Escola de Ciências e Tecnologia, Universidade de Évora, Évora , Portugal.

[c]CCMar - Centro de Ciências do Mar, Universidade do Algarve, Faro, Portugal.

[d]MARE – Marine and Environmental Sciences Center, Laboratório de Ciências do Mar, Universidade de Évora, Portugal .

*E-mail: sofia.cota-franco@newcastle.ac.uk

Phone: +44(0)191 208 6653

Fax: +44(0)191 208 7891

Address: School of Marine Science and Technology, Newcastle University, Ridley Building, Claremont Rd, Newcastle upon Tyne, NE1 7RU, United Kingdom.

Abstract

Pollicipes pollicipes (Crustacea; Pedunculata) is a delicacy on the Iberian Peninsula where, in recent years, stock shortages associated with high market value have increased interest in the aquaculture potential of this species. Though broodstock has been maintained in captivity, detailed culture conditions are lacking. The present study investigated the effects of rearing temperature on reproductive conditioning. During a 4-week period, broodstock were subjected to temperature regimes characteristic of stable spring temperatures (*spT*), increasing spring to summer temperatures (*sp-suT*) and increasing spring to summer temperatures with daily fluctuations of 1 ºC (*sp-suT2*). Broodstock were monitored for fecundity, egg lamella development and maturation, larval release rate, nauplius size and survival over 24 h. Cultured broodstock were fecund at smaller sizes (15.94 ± 0.23 mm RC) than wild-collected individuals at the beginning of the experiment (17.71 ± 0.65 mm RC). Fecundity increased significantly in all treatments and development of egg lamellae was highest in treatments in which the temperature increased over the experimental period (average 36% of mature egg lamellae in comparison to an initial 0%). Increasing temperature led to greater maturation of lamellae and more frequent spawning peaks. The number of nauplii released per aquarium (average 110 adult individuals) varied according to treatment and time, averaging 4670 ± 506 nauplii day^{-1}. Due to the low number of larvae released daily, it is suggested that adults might release larvae gradually, as embryos hatch within the mantle cavity. Average release rates increased towards the end of the conditioning period, with releases on peak days ranging from 10000 to 30000 nauplii per aquarium. For *spT*, peak values were observed in week 3, while *sp-suT* and *sp-suT2* showed peaks of release in weeks 2 and 4, when temperatures averaged 20 and 23 ºC, respectively. Temperature oscillations led to shorter intervals between peaks of release. In terms of

the larvae released, there were neither differences in numbers between treatments (128147 ± 13548 nauplii per aquarium over 28 days) nor in size of nauplii (202.89 ± 0.69 µm GW) or 24-h survival (91.56 ± 0.35%). Notwithstanding the need for further optimization, broodstock reproductive conditioning can be accomplished and a continuous supply of larvae obtained using the protocols described herein. Future studies should focus on the impact of food quality and photoperiod on reproductive conditioning, as well as the optimization of larval release induction protocols.

Keywords: Reproduction; barnacles; larva; aquaculture; temperature.

1. **Introduction**

Pollicipes pollicipes is a high-value barnacle species, historically subject to an intensive fishery (e.g. Freire and Garcia-Allut, 2000; Molares and Freire, 2003; Bald et al., 2006; Borja et al., 2006b; Jacinto et al., 2010). In recent years, growing concerns over depletion of natural stocks and the consequent stock protection measures (e.g. Borja et al, 2000, 2006a, 2006b; Castro, 2004; Jacinto et al., 2010) have increased interest in the potential of this species for aquaculture. However, there are few published reports that relate to culture conditions of *P. pollicipes* (e.g. Molares et al., 1994; Candeias, 2005; Cribeiro, 2007) and, as such, much remains unknown regarding broodstock conditioning, larval culture, settlement and juvenile grow-out.

P. pollicipes is found in clusters on the Atlantic coast from France to Senegal, in intertidal areas exposed to strong wave action (Stubbings, 1967; Barnes, 1996). These simultaneous hermaphrodites brood their developing embryos inside the mantle cavity (e.g. Molares, 1994; Cruz, and Araújo, 1999; Cruz, 2000; Pavón, 2003), until nauplii are released into the water column. Adults have been reported to produce approximately

30000 – 130000 embryos per batch and release asynchronously 1 to 5 batches per year during the breeding period from May to October (e.g. Cardoso and Yule, 1995; Cruz and Hawkins, 1998; Pavón, 2003; Macho, 2006).

Control over reproduction of *P. pollicipes* is essential for sustainable commercial aquaculture, allowing the extension of the production season by assuring the provision of larvae for culture and reducing the reliance on natural reproductive cycles. Embryos that are ready to hatch can be extracted from the adults in a process whose yield depends largely upon seasonal larval production, the synchrony of gonad development and individual egg lamella maturation. Other seedstock provision alternatives, such as from planktonic samples, are unreliable, due to the difficulty in quickly separating larvae to species, and artificial insemination appears to have limited potential for stalked barnacles (Walley et al., 1971; Lewis, 1975a; Qui et al., 1994). However, in spite of reports of broodstock maintenance and breeding in captivity (e.g. Kugele and Yule, 1996; Candeias, 2005; Cribeiro, 2007), detailed culture conditions and spawning frequencies are lacking.

For *P. pollicipes* in the wild, the effects of temperature on reproduction are hard to dissociate from latitude (e.g. Cruz, 2000) or seasonal food abundance (e.g. Molares, 1994; Cardoso and Yule, 1995; Cruz and Hawkins, 1998; Cruz and Araújo, 1999; Pavón, 2003), although temperature-regulated reproduction has been verified for *P. pollicipes* (Cardoso and Yule, 1995; Cruz and Hawkins, 1998). Studies on closely related *P. polymerus* in the wild have indicated that gonadal development and maturation are controlled more by temperature than by food (e.g. Cimberg, 1981; Page, 1983).

Previous reports from Candeias (2005) and Kugele and Yule (1996) with *P. pollicipes* broodstock strongly suggest that mating and spawning can be achieved in

captivity. However, spawning was sparse and collection of larvae sporadic. Other field studies on the species have focused on the timing of larval release in natural populations (e.g. Macho et al., 2005) and suggest that tidal cycle and photoperiod might be necessary triggering factors for spawning. Further observations in captivity on *Pollicipes polymerus* (Lewis, 1975b) support feeding as an inducer to the release of nauplii, as also seen in the well-studied annual breeder *Semibalanus balanoides*.

In addition, no studies involving rearing of *P. pollicipes* have imposed strict temperature controls or closely monitored the adult reproductive condition and larval quality (e.g. Cribeiro, 2007; Kugele and Yule, 1996). Further research into the effects of environmental factors on broodstock reproduction in captivity is warranted. Water temperature on the Atlantic coast of Portugal and Spain, where *P. pollicipes* is found, varies from 10 to 24 ºC, according to location and season (Instituto Hidrográfico, 2012; Meteo Galicia, 2012; Euskalmet, 2012) and its breeding period extends from March to September (e.g. Cardoso and Yule, 1995; Cruz and Hawkins, 1998; Pavón, 2003; Macho, 2006), which also coincides with the period of increased food abundance in the wild (e.g. Fiuza et al., 1982). Since elevated rearing temperatures are often used to induce reproduction in various cultured marine invertebrate species (e.g. bivalves; Loosanoff and Davies, 1950; Sastry, 1966) it is hypothesised here that this might also be appropriate to *P. pollicipes*.

The aim was to investigate the use of temperature for the reproductive conditioning of *P. pollicipes* and as a triggering factor for spawning in captivity, as well as establishing a viable reproductive conditioning protocol for this species. This study focuses on the effect of rearing temperature on the reproductive development of *P. pollicipes* broodstock by following fecundity index, egg lamella development index, release patterns, total number of nauplii released, their size and survival. Other factors,

such as adult growth and survival, were also monitored due to their relevance for assessment of reproductive fitness.

2. Materials and Methods

2.1. Stock collection and acclimatization

Clusters of *P. pollicipes* were collected from Cabo Sardão (37°36'24.70", -8°49'2.00", Portugal; 25th April, 2013) and transported in air within 3 h to the Ramalhete Aquaculture Station at the Centro de Ciências do Mar (37º00'22.39''N; 7º58'02.69''W, Faro, Portugal). Individuals were acclimated in aquaria for 14 days to stabilize mortality rates and adjust to the artificial feeding regime. During acclimatization (from 25th April to 8th of May, 2013) the clusters were kept in identical recirculating conditions of 16.3 ± 0.3 ºC and 36.6 ± 0.4 ppt. Prior to the experiment, clusters of barnacles of ≥ 5.0 mm rostro-carinal distance (RC) were weighed, photographed (Olympus © E-410) and counted. They were then mapped photographically within each cluster and measured for RC and stalk length (SL), using digital callipers. Clusters were assigned to particular groups with similar size-related population structures (adults with RCs ≥ 12.5 mm and juveniles with RCs of 5.0 - 12.55 mm), similar numbers and biomass.

2.2. Reproductive conditioning experiments

Barnacles were separated into 9 groups (146 ± 13 barnacles; 15.34 ± 4.76 mm RC; mean ± SD), of similar size-related population structures (11.81 ± 2.09 % 5.0 ≤ RC < 10.0 mm, 16.66 ± 2.57 % 10.0 ≤ RC < 12.5 mm, 53.71 ± 3.03 % 12.5 ≤ RC < 15.0 mm, and 29.63 ± 3.05 % RC ≥ 15.0 mm; mean ± SD) and distributed across 3 recirculating systems (each with 3 x 60 L aquaria; 1 group per aquarium). Over the following 4 weeks (from May 9th to June 6th, 2013), they were subjected to different temperature regimes. Temperature treatments were set-up as follows: (*spT*) constant spring

temperature of 16 °C (from day 1 to 28); (*sp-suT*) increase from spring temperatures 16 °C (on day 1) to summer temperatures 24 °C (on day 28); and (*sp-suT2*) increase in temperature from 16 °C (on day 1) to 24 °C (on day 28) with diel temperature fluctuations of ± 1 °C of temperature (Fig. 1). Temperature was controlled by an Aquatronic Aquarium Controller ACQ110©, with values programmed for each 3h, and monitored in each aquarium by Thermochron® iButton® DS1921G temperature data loggers. Each semi-open system had a total volume of 380 L, fully exchanged in 12 – 24 h (100 – 200 % renewal d^{-1}), including biological filtration.

In each aquarium, barnacles were attached to a net (1 cm^2/square mesh) and suspended at approximately half the height of the water column. Aquaria contained natural filtered seawater (FSW, 20 µm), with photoperiod programmed to follow the natural cycle, dim light intensity (100 – 200 lux), and turbulent conditions through the use of Hydor Koralia Pumps© and bottom aeration (Elite Air Stone, Rectangular, Extendable, 25 cm). Every day, aquaria were subjected to a tidal cycle of 3 h, during which time the water level decreased to half of the aquarium depth, leaving the barnacles exposed to air for approximately 2.5 h. Daily feeding included *Artemia* sp. (4 % dry weight per day; Artemia International GSL®), for 2 h prior to the start of the tidal cycle. *Artemia* cysts were hatched in 15-L conical flasks with FSW, 36 ± 1 ppt, 28 ± 1 ºC and strong bottom aeration (Sorgeloos and Persoone, 1975). After 24 h, *Artemia* nauplii were separated from the cysts and samples were counted to estimate daily feeding volumes. Dissolved oxygen, oxygen saturation and salinity, respectively 7.3 ± 0.1 mgO_2 l^{-1}, 98.9 ± 1.9 % and 36.5 ± 0.7 psu (mean ± SD), were measured daily pre-tidal cycle.

2.3. Data collection and treatment

The groups of barnacles in each aquarium were monitored for barnacle growth rate, survival rate, fecundity index, lamella development and naupliar release rates. Naupliar

size and survival over a 24-h period were also monitored. At days 1 and 28, mapped individuals were measured with digital callipers for RC and SL, with specific growth rate (SGR-RC; % 28 d^{-1}) calculated by [$SGR=(\ln((RC_{tf})/RC_{ti})/(tf-ti)\times100)$], where tf and ti are the initial and final days of experiment, respectively 0 and 28 and RC/SL ratio (RC/SL) calculated by [$RC/SL=RC_t/SL_t$], where t is time in days. Replicate aquaria were screened daily for dead individuals; which were carefully removed from clusters and measured for RC length, to estimate daily adult mortality rates (% d^{-1}, dM; where [$dM=(N_{dead}/N)\times100$], where N_{dead} is number of dead individuals and N is the total number of individuals), total survival (% $28d^{-1}$, tS) and average RC size of dead individuals (mm RC, dRC). Egg lamellae were removed from a sample of adult barnacles (n=30; RC $\geq$ 12.5 mm) to assess fecundity (FI; %) and maturity, by macroscopic analysis of lamella development stage index (LDSi), according to Table 1. Fecundity was calculated by [$FI=(N_{lamellae}/N)\times100$], where $N_{lamellae}$ is the number of individuals with lamellae and N is the total number of individuals. This was done at the beginning and end of the experimental period to compare the FI and LDSi of the initial broodstock (hereafter as *control*) and the experimental treatments, in addition to RC and RC/SL measures. Extracted egg lamellae were then left to hatch (in identical conditions to nauplii maintenance) and a number of released nauplii (n=30 nauplii) were maintained for 24 h to estimate naupliar size (GWn) and 24-h survival (24hS), as described below. Each day, all nauplii released from each aquarium were collected and counted (through volumetric sampling) to estimate daily (# day^{-1}, dRR), weekly (# $week^{-1}$, wRR) and total naupliar release rates (# $28d^{-1}$, tRR) per aquarium (where [$RR=n_{released}/N$], where RR is release rate, $N_{released}$ is number of released nauplii per aquarium and N is the total number of individuals per aquarium). Barnacle nauplii were collected using 80-µm filters placed over the outlet of each aquarium. Samples of 30

barnacle nauplii per treatment were maintained, to estimate survival to 24 h, in Petri dishes (50x9 mm; 12 mL; BD Falcon®), in FSW, 18 ± 1 ºC, 16:8 L/D (dim light) and 35 ± 1 ppt. To compare naturally released nauplii with extracted nauplii, all larvae were also photographed and measured (Image J®) for greatest width.

2.4. Statistical analyses

All statistical analyses were performed using STATISTICA 7.0®. Percentages (%) were arcsine transformed. Data were subjected to parametric tests including analysis of variance (ANOVA) or analysis of covariance (ANCOVA), with time as covariate, when assumptions for normality and homoscedasticity were met (Shapiro-Wilk and Levene tests, respectively). The significance level was set at α=0.05. Significant ANOVAs and ANCOVAs were followed by a Tukey test to identify differences among groups. Data that did not fulfil the assumptions for normality and homoscedasticity were subjected to non-parametric tests (Kruskal-Wallis test). Data in figures and tables are presented as mean ± standard error (SE), where not specified.

3. **Results**

3.1. SGR, RC/SL proportion and survival metrics

The average specific growth rate was 0.84 ± 0.16 % RC $28d^{-1}$ (Table 2), with no significant differences between treatments (ANOVA, F=0.10; P=0.90). However, differences were found for RC/SL (ANOVA, F=3.68; P=0.01). Collected broodstock had a RC/SL index of 1.28 ± 0.38. While individuals in *spT* and *sp-suT2* had higher RC/SL indices, *sp-suT* individuals had lower indices, comparable to the initial broodstock (Table 2). Significant differences were found between *sp-suT* and *sp-suT2* (Tukey Test; P=0.02), but not between the remaining treatments (Tukey test; P≥0.06).

Differences between treatments were not significant for daily mortality (ANOVA, F=1.77; P=0.17) and total survival (ANOVA, F=4.36; P=0.07; Table 2). Daily mortality averaged 0.38 ± 0.03 % d^{-1}, while total survival was 93.66 ± 0.76 % after 28 days (Table 2). The size of dead individuals in *spT* and *sp-suT* averaged 14.40 ± 0.34 mm RC, while in *sp-suT2* they were significantly different, measuring 11.91 ± 3.44 mm RC (ANOVA, F=6.76; P<0.01; Tukey Test, P<0.01).

3.2. Fecundity index and lamella development

The temperature regime significantly affected the fecundity index (Fig. 2a; ANOVA; F=11.35, P<0.01), with increasing temperatures stimulating breeding. Treatments *sp-suT2* and *spT* did not differ significantly from initial fecundity (Tukey Test; P≥0.30; 12.22 ± 4.00 and 14.44 ± 5.09 % fecundity, respectively), unlike *sp-suT* (Tukey Test; P<0.01). The fecundity index of broodstock reared at *sp-suT* was significantly higher than the other treatments (Tukey Test; P≤0.05) and individuals in this treatment reached 26.67 ± 1.92 % fecundity in just 4 weeks, equivalent to five times the fecundity at the start of the experiment, when 5.09 ± 0.08 % of individuals carried egg lamellae.

Temperature regime did not affect the size of individuals that bore egg lamellae (Fig. 3; ANOVA F=2.91, P=0.07), which averaged 15.94 ± 0.31 mm RC. Nevertheless, there were significant differences between the size of fecund and non-fecund individuals in the initial broodstock (ANOVA, F=8.11, P=0.01; Tukey Test, P=0.02), which were respectively 17.71 ± 0.65 mm RC and 15.37 ± 0.15 mm RC.

The initial lamella development index was 0.33 ± 0.21, increasing to 0.80 ± 0.36, 2.53 ± 0.47 and 3.15 ± 0.60 after 4 weeks of conditioning for *spT*, *sp-suT* and *sp-suT2*, respectively (Fig. 2b). Lamella development stage index was different according to conditioning regime (ANOVA, F=7.28, P<0.01), as *sp-suT* and *sp-suT2* were higher

than initial values (Tukey Test, P≤0.01). Between treatments, final lamella development index was only significantly different between *spT* and *sp-suT2* (Tukey Test, P=0.02). Broodstock initially had 80 % of stage 0 lamellae, and 20 % of stages 1 and 2 lamellae, with no mature egg lamellae observed at the beginning of the experiments (Fig. 4). After 28 d of rearing, *spT* had 60 % stage 0 and 40 % stage 1 and 2, while *sp-suT* and *sp-suT2* had only 36 % and 23 % of stage 0 lamellae, and 32 and 38 % of mature egg lamellae.

3.3. Naupliar release rates

Average daily release rates did not vary with treatment (ANOVA, F=1.14, P=0.32; table 3), averaging 4670.90 ± 506.63 nauplii d^{-1}, though they did with time (ANCOVA, F=2.74, P<0.01; Fig. 5a). Individuals in *spT* showed one peak release (≥10000 larvae) by week 3, while in *sp-suT* and *sp-suT2* there were two release peaks, in weeks 2 and 4 (Fig. 5a). The larval release rate of individuals reared at *spT* peaked at day 23 (15833 ± 2976 nauplii) (Fig. 5a; Tukey Test, P>0.01). Larval release rates for *sp-suT* peaked (Tukey Test, P>0.01) at day 11 (16531 ± 3937 nauplii) and day 28 (11969 ± 2246 nauplii), while for *sp-suT2* this happened at days 14 (13179 ± 7371 nauplii) and 27 (25806 ± 4677 nauplii).

Average release values increased with time and weekly rates were significantly different (Fig. 5b; ANOVA, F=35.43; P<0.01). Significantly lower numbers of larvae were released in week 1 (5310 ± 1891 nauplii $week^{-1}$) when compared to week 4 (66424 ± 9505 nauplii $week^{-1}$; Tukey Test, P<0.01). Average release rate did not differ with treatment (Tukey Test; P≥0.68), except in week 4 when *sp-suT* was significantly lower than both *sp-suT2* and *sp-suT* (Tukey Test; P=0.01), while these two did not differ from each other (Tukey Test; P=1.00).

Total release rates did not vary according to treatment (ANOVA, F=1.27, P=0.35). On average 126292 ± 2700 larvae were released per aquarium, although *sp-suT2* presented the highest values, followed by *spT* and *sp-suT*. Total numbers of nauplii released per aquarium were 125692 ± 20154, 113075 ± 10223 and 145674 ± 1129 for *spT*, *sp-suT* and *sp-suT2*, respectively (Table 3).

3.4. Nauplius size and 24-h survival

Size did not vary between hatched in vitro and naturally released nauplii (ANCOVA: F=0.01, p=0.99), which measured 202.89 ± 8.69 µm GW. Similarly, nauplius size was not significantly different among treatments (ANCOVA, F=0.03; p=0.97; Table 3). Nauplius 24-h survival averaged 91.56 ± 0.35 %, without differences between temperature regimes (ANOVA, F=0.06, p=0.94; Table 3).

4. **Discussion**

This study confirms that *P. pollicipes* can be temperature conditioned effectively in captivity in a relatively short period of time. Broodstock were collected in April 2013 and although about 5 % of the individuals were brooding eggs, none were carrying mature egg lamellae: on collection 80 % of egg lamellae were stage 0 and one month later up to 38 % had mature embryos. Although this might not allow exact estimation of the conditioning time required for later-season broodstock to develop from the post-reproduction state to reproductive state, it provides the first basis for reproductive conditioning in this species. In the present study, conditioning was studied for broodstock collected early in their natural spawning season. Hence, future studies could also focus on later-season broodstock. It may be of particular interest to extend the experimental periods with the aim of validating long-term broodstock conditioning. Rearing temperature affected broodstock conditioning, fecundity index, lamella

development index and daily release patterns, although there were no differences in total number of released nauplii, nauplius size and nauplius 24-h survival rate.

Broodstock SGR-RC did not differ significantly between treatments, varying between 0.73 and 0.93 % RC $28d^{-1}$, i.e. within the range observed in the wild (e.g. 0.17 – 0.66 mm RC $month^{-1}$; Cruz, 2000). No differences in mortality rates were found; the daily mortality rate was 0.34 % d^{-1} and mortality over 28 days was 6.44 ± 1.54 %, similar to field study results (Cruz, 2000, Goldberg, 1984), where average mortality is of the order of 4 % $month^{-1}$. In captive broodstock, mortality seems to occur mostly during the acclimatization period, as individuals on the external part of the clusters are often injured during collection. From previous experience, *P. pollicipes* adults and juveniles take between 1 and 2 weeks to acclimatize to feeding in captivity.

Unlike SGR and mortality rates, RC/SL differed between treatments, with higher RC/SL in *sp-suT2*, followed by *spT* and *sp-suT*, which was closer to the initial broodstock value, suggesting differences in the quality of growth with growing conditions. Stalk elongation in relation to RC has previously been observed in stock maintained in sub-optimal culture conditions (unpublished results), possibly due to increased competition, and has also been reported in the wild (Cruz, 2000). Individuals with a correspondingly lower RC/SL index generally had a less firm stalk. The fact that RC/SL did not decrease in any of the treatments with time suggests both a lack of competitive pressure (that results in stalk elongation) and conditions that were limiting for development.

Temperature, as well as food availability, is known to affect the reproductive cycles of many barnacle species, such as *P. pollicipes*, *P. polymerus*, *Chthamalus depressus*, *Capitulum mitella*, *Chthamalus fissus* (Cimberg, 1981; Cruz and Hawkins, 1998; Hines, 1978; Page, 1983; Patel and Crisp, 1960ab). The regulatory effect of temperature on

reproduction is both metabolic and physiological, and can affect gonadal maturation and development rate, mating, brooding and larval release. The three treatments were selected in order to allow comparisons between temperature regimes, from early season temperature (*spT*) to reproductive season temperatures, with (*sp-suT2*) or without (*sp-suT*) daily fluctuations. In spite of the lack of previous conditioning data for *P. pollicipes*, several temperature cycles have been used for barnacles, from a broad range of stable temperatures (e.g. Leung, 2002), to stable but higher temperatures (e.g. Patel, 1959; Patel and Crisp, 1960b) or lower temperatures for species inhabiting temperate or cold waters (e.g. Crisp, 1957). Results from studies on bivalves show that regimes of increasing temperature can help induce breeding (e.g. Loosanoff and Davies, 1952; Chavez-Villalba et al., 2002). Reproductive cycles are often characterized by brooding frequency and the pattern of gonad development (e.g. Cardoso and Yule, 1995; Cruz and Araújo, 1999). Although gonadal development allows a more accurate estimation of reproductive development, its application is limited in terms of day-to-day assessment of reproductive state under culture conditions. Furthermore, individual maturity does not imply successful mating and brooding, and therefore the presence of egg lamellae, or released larvae, are important for assessing reproductive state.

Fecundity index increased in all treatments, from 7 to 22 % in 4 weeks, compared to the initial broodstock fecundity. The best results were for *sp-suT* where 27 % fecundity was recorded. Successful conditioning studies with other barnacle species have achieved 20 – 100 % fecundity in 2 to 6 weeks, depending on species and temperature (e.g. Patel and Crisp, 1960b). However, conditioning studies with *C. mitella* (e.g. Leung, 2002) could not achieve fecundity above 5 % for in-season individuals (or 0 % for off-season individuals) even after 6 weeks of conditioning, independent of the temperatures tested. In the present case, not only did the fecundity increase significantly, but it reached

values comparable to wild populations of *P. pollicipes* within 28 days of rearing. Fecundity indices in the wild are reported to vary greatly (0 – 80 %, Cruz, 2000), with high inter-annual and site-specific variation (Pavón, 2003, Cardoso and Yule, 1995). Other factors, such as tidal height, can account for differences from 60 – 100 % fecundity in the intertidal to as low as 5 % fecundity in the subtidal, during the breeding season (Cruz and Araújo, 1999). Peak values in the wild were recorded from May to September (18 – 80 %) by Cruz (2000) and August to October (20 – 50 %) by Molares (1994). Consistent with the broodstock collection results of the present study, Cruz (2000) also noted that *P. pollicipes* fecundity was higher in large individuals (RC > 15 mm; 40 – 80 %) than in smaller ones (12 < RC ≤ 15 mm; 15 ‑ 40 %).

The lamella development index increased significantly in *sp-suT* and *sp-suT2*, transitioning from, on average, stage 0 to stage 3. Furthermore, the initial 0 % mature lamellae found, increased to between 32 and 38 % in *sp-suT* and *sp-suT2*, respectively, although in *spT* mature lamellae remained at 0 %. This, together with fecundity results, indicates that within 4 weeks lamellae cannot only develop from fully immature to ready to hatch, but also non-fecund individuals can mate and develop lamellae in that period if conditions are favourable. Interestingly, although *spT* showed an increase in fecundity, there was no increase in lamella maturity, which suggests not only that brooding time might be significantly reduced in the higher temperature treatments, but that there might also be a limiting temperature that triggers gonadal development. Nevertheless, the small increase in fecundity indicated that even in the absence of temperature increase, reproductive development can still occur, albeit at a slower rate.

Broodstock fecundity increased by 6 % in *sp-suT2* and *spT* and by about 21 % in *sp-suT*, in the space of 28 days. However, when lamella development stage at day 28 is considered, *sp-suT* and *sp-suT2* showed a significantly higher number ready to hatch;

between 32 and 38 % higher compared to *spT*. When total release rates were compared between treatments, *sp-suT2* released in total 14 % more nauplii than *spT* and 23 % more than *sp-suT*. Under these conditions, the treatment with highest fecundity was *sp-suT*, while the treatment with highest total release rate was *sp-suT2*. This raises the question of whether it is better to rear apparently highly fecund individuals that release fewer larvae, or less fecund individuals that release more larvae. These observations may be explained by the fact that the sampling frequency for assessment of fecundity occurred only at day 0 and day 28, while releases of larvae occurred throughout and were monitored daily. It is important to note, however, that no differences in 24-h larval survival were found between releases of different treatments, though later differences in viability cannot be excluded. Therefore, while daily release rates provide a trend in time, fecundity index and lamella development stage provide a snapshot of reproductive fitness pre- and post-conditioning. One could argue either that the higher fecundity of *sp-suT* indicates a higher propensity for release of nauplii, or that the lowest fecundity of *sp-suT2* at the end of the experiment is due to a previously high release rate during the experimental period. Considering that egg incubation for embryonic development is thought to take between 15 to 24 days (Molares, 1994; Cruz, 2000), if nauplii had been released within that conditioning period it is unlikely that there would be sufficient time to deposit new lamellae.

Similar daily release rates were recorded across treatments, averaging approximately 4500 nauplii day^{-1}, although peak values were frequently above 10000 nauplii day^{-1}. These values may seem low compared to previous studies, e.g. 16229 $\pm$ 1094 and 34172 $\pm$ 1807, according to barnacle size (Cruz and Araújo, 1999; Cruz, 2000) compared to 15547 ± 2589 embryos per lamella in the present study. However, when lamellae were left to hatch over a 24-h period, hatching rate was about 15 % increasing

to 25 % by 48 h. Embryos at the periphery of the egg lamella were often several development stages ahead of the central embryos and hatched between 2 – 10 days earlier. This is probably due to differences in oxygenation between central and peripheral embryos (Crisp, 1959). Although these observations relate to isolated egg lamellae hatched in vitro, lamellae brooded inside the mantle cavity of the adults might be subject to similar conditions with partial hatching occurring over time. Indeed, lamellae collected from wild individuals did contain embryos at different stages of development. Therefore, it is hypothesised that adults carrying mature egg lamellae may release their newly hatched nauplii over a period of days, rather than as a single event. Other barnacle species, such as *Semibalanus balanoides*, have been observed to retain their embryos after reaching a hatch-ready state for at least a month until conditions are suitable for release (Clare et al., 1982). This would explain the low daily release rate associated with comparatively high fecundity index and a high percentage of lamellae with embryos that are ready to hatch in the increasing temperature treatments.

Given the experimental design, it was not possible to estimate the number of adults releasing larvae. This would have provided valuable insight into the proportion of mature adults and actively releasing individuals and, consequently, the number of adults needed to ensure a suitable volume of production and maintain a diverse larval genetic background. This could be addressed by studies following single individuals and not cohorts which, despite being more applicable to aquaculture, have experimental limitations.

For *sp-suT*, release rates seemed to increase and peak at day 11 (19.6 °C), followed by a decrease until almost day 28 (23.0 °C) when they increased again. On the other hand, for *sp-suT2*, the same behaviour was observed, but peaks occurred at days 14 and 27, where temperatures averaged 20.1 °C and 22.6 °C. In both treatments, peak releases

started as they approached 20 °C, indicating that this temperature might be relevant to trigger reproduction. It is hypothesised that this temperature might be suitable for a constant high-temperature conditioning regime, and further studies should investigate this, as it could imply a commercially more cost-effective conditioning regime than increasing temperatures. These patterns differed between groups in terms of peak frequency, between stable and increasing temperatures, but also the timing and intensity of the peaks in treatments kept at increasing temperature.

Although the two treatments of increasing temperatures peaked at similar times and at similar intensities, *sp-suT2* showed a higher average dRR and less time between dRR peaks compared to *sp-suT*. The effects of temperature variations are still poorly understood and the relative importance of temperature for different species may be related to temperature intensity, critical values, or even the effect on development rates (Muranaka and Lannan, 1984; Cardoso and Yule, 1995). The reduced time between dRR peaks in *sp-suT2* suggests that daily changes of temperature, as would be experienced in the wild, induce faster development than other tested conditions. Temperature cycles are reproduction regulators, being advantageous for allowing the synchronization of reproduction between individuals, and essential when female and male development does not occur contemporaneously. In general, for *P. pollicipes*, it is accepted that the increase in temperature triggers reproduction, and this is supported by the present results, although in the wild this occurs in synchrony with the increase in food availability. For *S. balanoides*, the gonadal maturation is triggered by both decreased temperature and photoperiod (Barnes, 1963, 1989; Crisp, 1986), while for *Chthamalus stellatus*, *Elminius modestus*, *Balanus perforatus* and *B. amphitrite*, temperature and food affect gonad development (Patel and Crisp, 1960b). *P. pollicipes* may differ, however, as the maturation of female and male gonads is distinct throughout

the year. Cruz (2000) observed that during the breeding season, the percentage of mature female gonads ranged between 20 and 60 %, while the seminal vesicles of most of the population were mature all year round. Molares (1994) suggested that the maturation of the female gonad restricts reproduction to the period between March and September. In the absence of limiting conditions, however, it is likely that gonad recovery occurs at a faster pace, allowing for shorter brooding periods and therefore multiple release peaks. Under this scenario, temperature variations might effectively act as release triggers, leading to closer release peaks and to higher intensity spawning, as seen in *sp-suT2* in comparison to *sp-suT*.

Nauplii collected from the adults and from natural releases were analysed further for size and 24-h survival rate, but no differences were found between treatments and between extracted and collected nauplii. These are promising results for nauplii released in culture, since naupliar survival does not seem to be affected by source or adult rearing conditions. Nevertheless, it cannot be excluded that wild-raised and naturally-released larvae might show differences in health as larvae develop. Further studies should evaluate possible effects on cyprid metamorphosis, survival and larval settlement, considering in particular larvae obtained by extraction from wild adults vs. larvae collected from releases in culture. To our knowledge, no report of such effects has been observed in barnacle species, although the larval period of other species (e.g. *Balanus improvisus*) can be significantly extended by dietary deficiency, yet still result in ostensibly healthy larvae.

5. **Conclusions**

Temperature conditioning of *P. pollicipes* can be achieved in culture in less than a month, provided that individuals are previously acclimated. Better results for

conditioning, in terms of fecundity, percentage of mature lamellae and frequency of larval release peaks were achieved for broodstock reared at increasing temperature. It is suggested that increasing temperature might decrease development time and accelerate egg lamella maturation, as food availability was not limiting. This would allow for multiple release peaks. Furthermore, broodstock kept with daily temperature variations showed slightly better results in terms of lamella development index, lamella maturation and time between peaks of release. However, no significant differences were noted between the total number of larvae produced, suggesting that the conditioning effect mostly relates to timing and concentration of release events. Interestingly, the daily average number of nauplii released was below that expected, given the number of embryos produced by each adult. Therefore it is proposed that individuals might be releasing larvae of the same brood over a period of days, as the embryos hatch within the mantle cavity. Furthermore, analysis of naupliar size and 24-h survival did not reveal differences between extracted and released larvae, validating both protocols.

The present work establishes a rearing protocol for broodstock that allows for maintenance in culture and spawning stimulation, and that can serve as a basis for future broodstock reproduction in captivity. Furthermore, the proposed conditioning method could be easily translated to a commercial aquaculture setup. The results from the present study support the proposal that *P. pollicipes* conditioning can be a valuable tool for larval collection in captivity and production of larvae under culture conditions, not limited to the breeding season, and in significant numbers for scaling up cultures.

ACKNOWLEDGEMENTS

This work was supported by the Fundação para a Ciência e Tecnologia, research grant SFRH/BD/63998/2009 to S.C. Franco, financed by the Programa Operacional Potencial

Humano (POPH – QREN) and co-financed by the European Social Fund (ESF, European Union)) and to A.V. Sykes, research grant SFRH/BPD/36100/2007 and Programa Investigador FCT 2014 (IF/00576/2014). Further support has been received from by the 7th Framework Programme (FP7/2007-2013), grant n° 227799-ASSEMBLE, to A.S. Clare and S.C. Franco, for development of research in CCMar facilities. The authors would further like to thank CCMar for hosting the present research and to João Reis and Miguel Viegas for invaluable technical support. N. Aldred and S.C. Franco acknowledge funding support from Office of Naval Research Grants N00014-08-1-1240 and N00014-13-1-0634 to ASC and N00014-13-1-0633 to ASC and NA. The authors would further like to thank A.P. Cribeiro for his support and to the anonymous reviewers of this manuscript, for their valuable comments.

References

Bald, J., Borja. A., Muxika, I., 2006. A system dynamics model for the management of the gooseneck barnacle (*Pollicipes pollicipes*) in the marine reserve of Gaztelugatxe (Northern Spain). Ecol. Model. 194, 306-315.

Barnes, H., 1963. Light, temperature and the breeding of *Balanus balanoides.* J. Mar. Biol. Assoc. UK. 43(3), 717-727.

Barnes, M., 1989. Egg production in cirripedes. Oceanogr. Mar. Biol. Annu. Rev. 27, 91-166.

Barnes, M., 1996. Pedunculate cirripedes of the genus *Pollicipes*. Oceanogr. Mar. Biol. Annu. Rev. 34, 303–394.

Borja, A., Valencia, V., Castro, R., Franco, J., Bald, J., Uriarte, A., Mendizabal, M., Aguirrezabalaga, F., 2000. Establecimiento de las bases tecnicas de conocimiento del area de San Juan de Gaztelugatxe con vistas a su posible declaracion como reserva marina. GV Informes Técnicos (Departamento de Agricultura y Pesca). 87: 1-152.

Borja, A., Liria, P., Muxika, I., Bald, J., 2006a. Relationships between wave exposure and biomass of the goose barnacle (*Pollicipes pollicipes*, Gmelin, 1790) in the Gaztelugatxe Marine Reserve (Basque Country, northern Spain). ICES J. Mar. Sci. 63, 626-636.

Borja, A., Muxika, I., Bald, J., 2006b. Protection of the goose barnacle *Pollicipes pollicipes*, Gmelin, 1790 population: the Gaztelugatxe Marine Reserve (Basque Country, northern Spain). Sci. Mar. 70, 235-242.

Candeias, A.T., 2005. Aspects of feeding in barnacle larvae (Cirripedia: Thoracica). PhD Thesis. University College of Wales, Menai Bridge, UK.

Cardoso, A., Yule, A., 1995. Aspects of the reproductive biology of *Pollicipes pollicipes* (Cirripedia; Lepadomorpha) from the southwest coast of Portugal. Aquat. Ecol. 29, 391-396.

Castro, J., 2004. Predação humana no litoral rochoso alentejano: caracterização, impacte ecológico e conservação. PhD Thesis. Universidade de Évora, Évora, Portugal.

Chávez-Villalba, J., Pommier, J., Andriamiseza, J., Pouvreau, S., Barret, J., Cochard, J.C., Le Pennec, M., 2002. Broodstock conditioning of the oyster *Crassostrea gigas:* origin and temperature effect. Aquaculture. 214(1), 115-130.

Cimberg, R.C., 1981. Variability in brooding activity in the stalked barnacle *Pollicipes polymerus*. Biol. Bull. 160, 31-42.

Clare, A.S., Walker, G., Holland, D.L., Crisp, D.J., 1982. Barnacle egg hatching: a novel role for a Prostaglandin-like compound. Mar. Biol. Lett. 3,113-120.

Cribeiro, A.P., 2007. Explotacion del percebe, *Pollicipes pollicipes* (Gmelin, 1789), na costa do Ortegal. influencia dos factores abioticos, estudo en condicions experimentais da estrutura poboacional. MSc Thesis. Universidade de Santiago de Compostela, Santiago de Compostela, Spain.

Crisp, D.J. 1957. Effect of low temperature on breeding of marine animals. Nature. 179, 1138-1939.

Crisp, D.J., 1959. Factors influencing the time of breeding of *Balanus balanoides*. Oikos. 10(2), 275-289.

Crisp, D.J., 1986. A comparison between the reproduction of high- and low-latitude barnacles, including *Balanus balanoides* and *Tetraclita* (*Tesseropora*) *pacifica*. ca., in

Cruz, T., 2000. Biologia e ecologia do percebe *Pollicipes pollicipes* (Gmelin, 1790) no litoral sudoeste português). PhD Thesis. Universidade de Évora, Évora, Portugal.

Cruz, T., Araújo, J., 1999. Reproductive patterns of *Pollicipes pollicipes* (Cirripedia: Scalpellomorpha) on the Southwestern coast of Portugal. J. Crust. Biol. 19, 260-267.

Cruz, T., Hawkins, S.J., 1998. Reproductive cycle of *Pollicipes pollicipes* at Cabo de Sines, South-West coast of Portugal. J. Mar. Biol. Assoc. UK. 78, 483-496.

Euskalmet, 2012. Agencia Vasca de Meteorologia. In www.euskalmet.euskadi.net. Accessed 2 February 2012.

Fiuza, A.F.G., Macedo, M.E., Guerreiro, M.R. 1982. Climatological space and time variation of the Portuguese coastal upwelling. Ocean Acta. 5(1), 31-40.

Freire, J., Garcia-Allut, A. 2000. Socioeconomic and biological causes of management failures in European artisanal fisheries: the case of Galicia (NW Spain). Mar Pol. 24, 357-384.

Goldberg, H., 1984. Posibilidades de cultivo de percebe, *Pollicipes cornucopia* Leach, en sistemas flotantes. Informes Técnicos del Instituto Español de Oceanografía. 19, 1-13.

Hines, A.H., 1978. Reproduction in three species of intertidal barnacles from central California. Biol. Bull. 154, 262-281.

Instituto Hidrográfico, 2012. Instituto Hidrográfico. In www.hidrografico.pt: Accessed 2 February 2012.

Jacinto, D., Cruz, T., Silva, T., Castro, J.J., 2010. Stalked barnacle (*Pollicipes pollicipes*) harvesting in the Berlengas Nature Reserve, Portugal: temporal variation and validation of logbook data. ICES J. Mar. Sci.: J. Conseil. 67: 19-25.

Kugele, M., Yule, A.B., 1996. The larval morphology of *Pollicipes pollicipes* (Gmelin 1970) (Cirripedia: Lepadomorpha) with notes on cyprid settlement. Sci. Mar. 60, 469-480.

Leung, T.Y., 2002. The ecology and reproductive biology of two intertidal barnacles. Mol. Ecol. 7, 1053-64.

Lewis, C.A., (1975b). Some observations on factors affecting embryonic and larval growth of *Pollicipes polymerus* (Cirripedia: Lepadomorpha) in vitro. Mar. Biol. 32, 127-139.

Lewis, C.A., 1975a. Development of the gooseneck barnacle *Pollicipes polymerus* (Cirripedia: Lepadomorpha): Fertilization through settlement. *Mar. Biol.* 32: 141-153.

Loosanoff, V.L., Davis, H.C., 1950. Conditioning of *V. mercenaria* for spawning in winter and breeding its larvae in the laboratory. Biol. Bull. 98, 60-65.

Loosanoff, V.L., Davies, H.C., 1952. Temperature requirements for maturation of gonads in Northern oysters. Biol. Bull. 103(1), 80-96.

Macho, G.M. 2006. Ecologia reproductiva y larvaria del percebey otros cirripeios en Galicia. In *Departamento de Ecoloxia e Bioloxia Animal, Facultade de Ciencias*. Vigo (Spain): University of Vigo, pp. 300.

Macho, G., Molares, J., Vázquez, E., 2005. Timing of larval release by three barnacles from the NW Iberian Peninsula. Mar. Ecol. Prog. Ser. 298, 251-260.

Meteo Galicia, 2012. Meteo Galicia. In www.meteogalicia.es. Accessed 2 February 2012.

Molares, J., 1994. Estudio del ciclo biologico del percebe (*Pollicipes cornucopia* Leach) de las costas de Galicia. Xunta de Galicia, Santiago.

Molares, J., Tilves, F., Pascual, C., 1994. Larval development of the pedunculate barnacle *Pollicipes cornucopia* (Cirripedia: Scalpellomorpha) reared in the laboratory. Mar. Biol. 120, 261-264.

Molares, J., Freire, J., 2003. Development and perspectives for community-based management of the goose barnacle (*Pollicipes pollicipes*) fisheries in Galicia (NW Spain). Fish. Res. 65, 485-492.

Muranaka, M.S., Lannan, J.E., 1984. Broodstock management of *Crassostrea gigas*: Environmental influences on broodstock conditioning. Aquaculture. 39(1), 217-228.

Page, H.M., 1983. Effect of water temperature and food on energy allocation in the stalked barnacle, *Pollicipes polymerus* Sowerby. J. Exp. Mar. Biol. Ecol. 69, 189-202.

Patel, B., 1959. The influence of temperature on the reproduction and moulting of *Lepas anatifera* L. under laboratory conditions. J. Mar. Biol. Assoc. UK. 38, 589-597.

Patel, B., Crisp, D.J., 1960a. Relation between the breeding and moulting cycles in cirripedes. Crustaceana. 2, 89-107.

Patel, B., Crisp, D.J., 1960b. The influence of temperature on the breeding and the moulting activities of some warm-water species of operculate barnacles. J. Mar. Biol. Assoc. UK. 39, 667-680

Pavón, C., 2003. Biologia y variables poblacionales del percebe, *Pollicipes pollicipes* (Gmelin, 1790) en Asturias. PhD Thesis. Universidad de Oviedo. Oviedo, Spain.

Qiu, W., Lin, G., Qi, Q., 1994. Development of *Pollicipes mitella* (Cirripedia: Scalpelidae): fertilization through hatched. J. Fujian Teach. Univ. Nat. Sci. 10(1), 95-100.

Sastry, A.N., 1966. Temperature effects in reproduction of the bay scallop, *Aequipecten irradians* Lamarck. Biol. Bull. 130, 118-133.

Sorgeloos, P., Persoone, G., 1975. Technological improvements for the cultivation of invertebrates as food for fishes and crustaceans. II. Hatching and culturing of the brine shrimp, *Artemia salina L.* Aquaculture. 6(4), 303-317.

Stubbings, H.G., 1967. The cirriped fauna of tropical West Africa. Bull. Brit. Mus. Nat. Hist.. 15, 229-319.

Walley, L.J., White, F., Brander, M., 1971. Sperm activation and fertilization in *Balanus balanoides*. J. Mar. Biol. Assoc. UK. 51, 489-494.

LIST OF FIGURES

Fig. 4 Percentage of egg lamellae according to development stage, extracted from broodstock reared under different temperature regimes. Stages were classified as (0) undifferentiated, (1) early differentiation, (2) mid differentiation, (3) late differentiation, (4) differentiated, according to Table 1. Stage 4 lamellae were ready to hatch, and nauplii would swim freely upon egg lamella membrane rupture. Treatments were as follows: (spT) constant spring temperature of 16 °C, (sp-suT) linear temperature increase from spring 16 °C (on day 1) to summer 24 °C (on day 28) and (sp-suT2) average increase in temperature from spring 16 °C (on day 1) to summer 24 °C (on day 28) with diel fluctuations of ± 1 °C. The values referring to the initial broodstock collected are presented for comparative purposes and listed as (Control).

Fig. 5 (a) Daily release rates, i.e. number of nauplii released per aquarium per day, and (b) weekly release rates, i.e. number of nauplii released per aquarium per week, during 4 weeks of conditioning and according to temperature regime. Treatments were as follows: (spT) constant spring temperature of 16 °C, (sp-suT) linear temperature increase from spring 16 °C (on day 1) to summer 24 °C (on day 28) and (sp-suT2) average increase in temperature from spring 16 °C (on day 1) to summer 24 °C (on day 28) with diel temperature fluctuations of ± 1 °C.

Figures

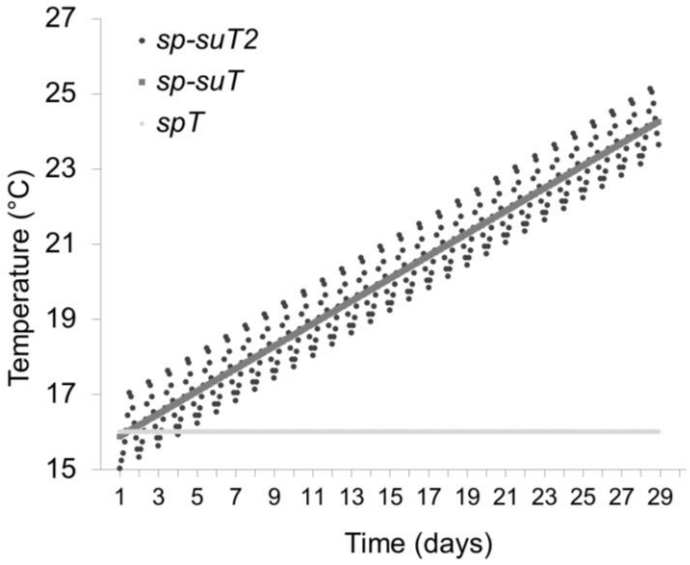

Fig. 1

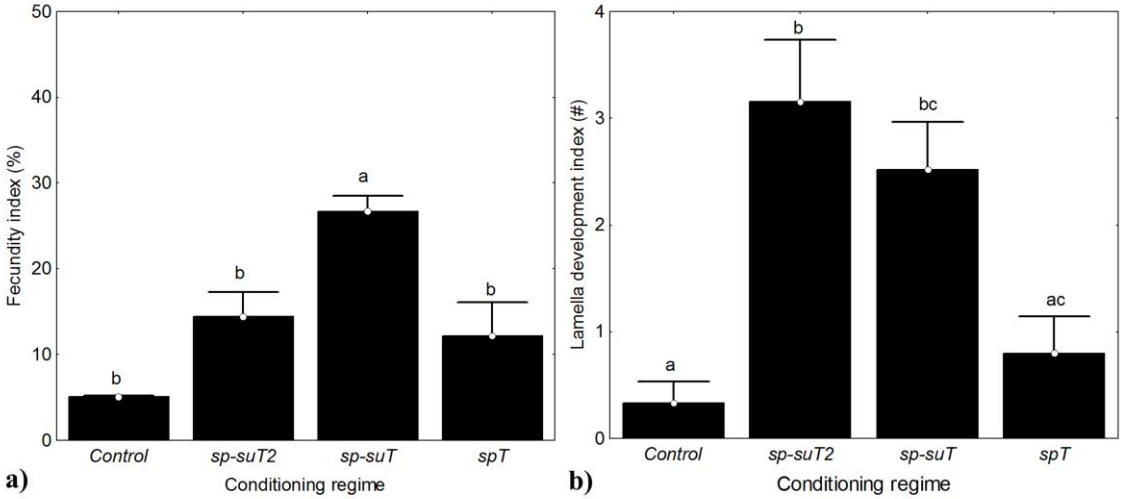
Fecundity index (%)
50
40
30
20
10
0
b
b
a
b
Control
sp-suT2
sp-suT
spT
Conditioning regime
a)
Lamella development index (#)
4
3
2
1
0
a
b
bc
ac
Control
sp-suT2
sp-suT
spT
Conditioning regime
b)

Fig. 2

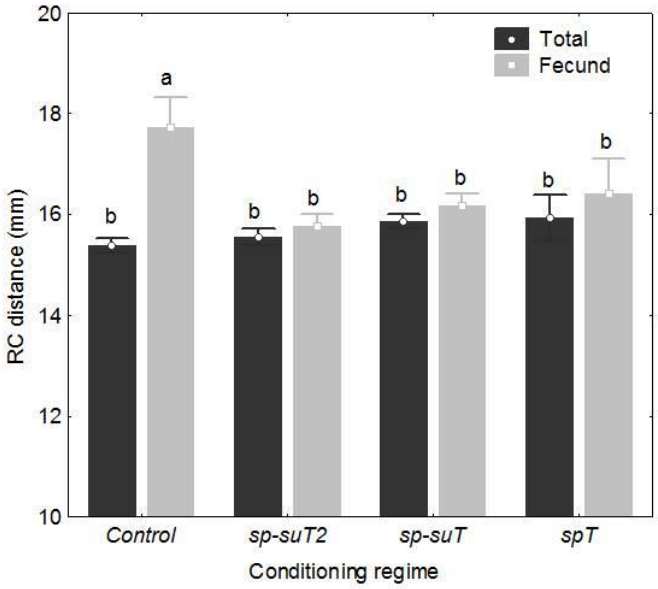
20
18
16
14
12
10
RC distance (mm)
Total
Fecund
a
b
b
b
b
b
b
b
Control
sp-suT2
sp-suT
spT
Conditioning regime

Fig. 3

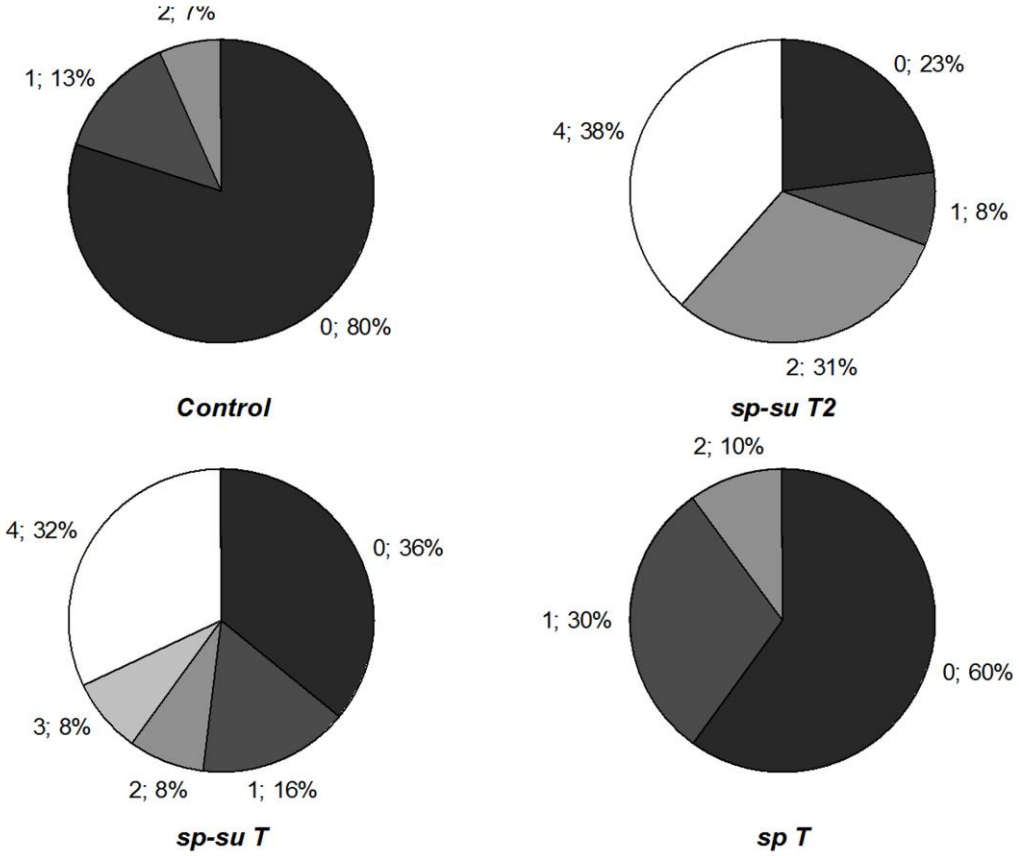
2; 7%
1; 13%
0; 80%
Control
0; 23%
4; 38%
1; 8%
2: 31%
sp-su T2
4; 32%
0; 36%
3; 8%
2; 8%
1; 16%
sp-su T
2; 10%
1; 30%
0; 60%
sp T

Fig. 4

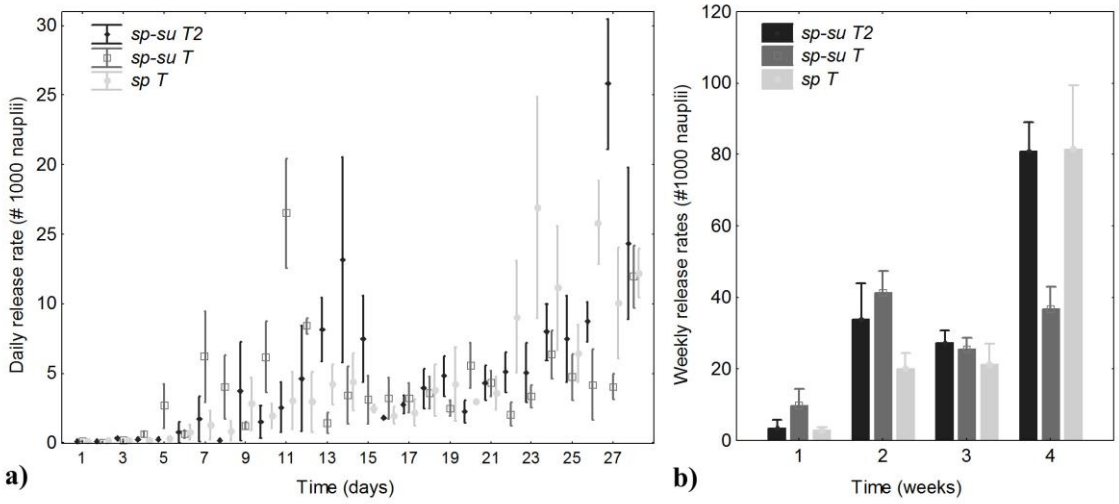
sp-su T2
sp-su T
sp T
Daily release rate (# 1000 nauplii)
Time (days)
a)
sp-su T2
sp-su T
sp T
Weekly release rates (#1000 nauplii)
Time (weeks)
b)

Fig. 5

Tables

Table 1. Criteria for the classification of lamellae according to development stage. Lamellae were analyzed macroscopically and classified according to colour and texture. Microscopical analysis was also done according to nauplii maturation stage, considering the differentiation stage of the eyes, appendages and digestive system. These were classified as *U* undifferentiated, *pD* partially differentiated and *D* differentiated.

Class	Development stage	Colour	Texture	Embryo development		
				Eye	Appendages	Stomach
0	Undifferentiated	Pink	Rigid	U	U	U
1	Early differentiation	Pink	Rigid	pD	U	U
2	Mid differentiation	Pink-Yellow	Semi-rigid	D	U	U
3	Late differentiation	Yellow-Brown	Flaccid	D	D	U
4	Differentiated	Yellow-Brown	Cloudy	D	D	D

Table 2. Growth and survival metrics for *P. pollicipes* adults grown for 4 weeks under different temperature regimes (*spT, sp-suT* and *sp-suT2*). Growth metrics considered included specific growth rate based on rostro-carinal distance (*SGR-RC*, % RC $28d^{-1}$) and proportion between rostro-carinal distance and stalk length (*RC/SL, #*; $RC/SL_{Control}$ =1.28 ± 0.38). Survival metrics included daily mortality (dM, % d^{-1}), total survival (tS, %) and rostro-carinal distance of dead individuals (RC_d, mm RC). Different letters within rows indicate significant differences (P<0.05).

	sp-suT2	*sp-suT*	*spT*
SGR-RC (% $28d^{-1}$)	0.83 ± 0.24 [a]	0.93 ± 0.31 [a]	0.73 ± 0.29 [a]
RC/SL (#)	1.47 ± 0.40 [b]	1.29 ± 0.29 [a]	1.37 ± 0.38 [ab]
dM (% d^{-1})	0.42 ± 0.56 [a]	0.29 ± 0.53 [a]	0.43 ± 0.54 [a]
tS (% 28d)	91.33 ± 1.63 [a]	94.98 ± 2.27 [a]	91.68 ± 0.76 [a]
RCd (mm RC)	11.91 ± 3.44 [a]	14.54 ± 2.76 [b]	14.27 ± 3.04 [b]

Table 3. Daily release rates (dRR, # nauplii aquarium d^{-1}), total release rates (tRR, # nauplii aquarium $28d^{-1}$), released nauplii I size (TL, µm) and nauplii survival after 24h (24hS; %), according to temperature regimes (*spT, sp-suT* and *sp-suT2*). Different letters within rows indicate significant differences (P<0.05).

	sp-su T2	*sp-su T*	*sp T*
dRR (larvae d^{-1})	4972.00 ± 722.15 [a]	4062.83 ± 466.15 [a]	4495.92 ± 635.55 [a]
tRR (larvae)	145674.51 ± 11292.40 [a]	113075.34 ± 10223.32 [a]	125692.33 ± 20154.10 [a]
GW (µm)	203.67 ± 2.84 [a]	202.33 ± 2.85 [a]	202.67 ± 5.90 [a]
24hS (%)	91.01 ± 1.52 [a]	92.00 ± 1.53 [a]	91.67 ± 2.91 [a]